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Original Research

Decreased annexin a2 (anxa2) levels in children with atopic dermatitis: A case-control study

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Abstract

Aim: Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in children. The pathogenesis of AD has not been clearly understood. The role of annexin A2 (ANXA2), which is an anti-inflammatory mediator, has not been investigated in the pediatric population with AD. The aim of this study is to investigate serum ANXA2 levels in children with AD.

Material and Methods: Three groups were enrolled in this study; an SPT-Pos group (skin prick test positive 25 subjects with AD), an SPT-Neg group (skin prick test negative 25 subjects with AD), and a control group (27 healthy subjects). The serum ANXA2 levels were measured using enzyme-linked immunosorbent assay (ELISA).

Results: We observed significantly lower serum ANXA2 levels in the disease group than in the control group [0.53 (range 0.14 - 4.38) pg/mL vs 0.94 (range 0.40 - 8.74) pg/mL, respectively; p = 0.01], especially in the SPT-Post group. However, there was no correlation among the parameters of ANXA2, IgE, eosinophil counts.

Discussion: ANXA2 may have the role of an anti-inflammatory mediator in the pathogenesis of AD in children. The ANXA2-associated pathways may be considered in the development of novel therapeutic approaches for the treatment of patients with AD.

Keywords

Annexin A2; Atopic Dermatitis; Children

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects 1-3 % of adults and 15-20 % of children worldwide. The incidence of AD has increased over the last decades worldwide [1-2]. The pathogenesis of AD has not been clearly understood. It has been accepted that complex interactions between environmental and genetic factors, such as epidermal barrier dysfunctions, cause chronic dermal inflammation [3]. Phospholipids are catalyzed by phospholipases A2 (PLA2) enzymes that are involved in many pathophysiological processes, such as inflammation and eicosanoid production. Also, the activity of phospholipase A2 was reported to be involved in inflammatory skin diseases such as atopic dermatitis and psoriasis [4]. Annexins (ANXs) are a family of calcium-dependent membrane-binding pro¬teins that perform several different functions, such as membrane trafficking, ion channel regulation, and anticoagulant activities, as well as an anti-inflammatory effect. Twelve ANXs have been identified in verte¬brates. They are mainly localized in the cytosolic milieu, they can also be found in the extracellular milieu which has anticoagulant and anti-inflammatory effects [5]. It has been demonstrated that some of ANXs (ANX-1, -2, and -5) have strong anti-inflammatory effects [6]. Also, it was demonstrated that some of ANXs (ANX-5 > ANX-2 > ANX-1) have inhibition of PLA2 activities in the dermo-epidermal area [7]. The aim of this study was to investigate serum ANXA2 levels in SPT-Pos, SPT-Neg, and control groups.

Material and Methods

This prospective study was performed in the Maternity and Children's Hospital, Batman, Turkey. In this study, AD was diagnosed based on standard Hanifin and Rajka criteria [8]. A total of 72 children aged 3 months - 3 years were enrolled in the study. Fifty patients with AD were divided into either the SPT-Pos (25 subjects) or the SPT-Neg group (25 subjects). The control group comprised of 27 healthy children. Children with infectious diseases, such as upper or lower airway infections within the four weeks prior to the study, were excluded. At the presentation, the children underwent skin prick testing (SPT) on the upper back. The SPT kit (Allergopharma, Reinbek, Germany) tested 10 antigens including two aero-allergens (Dermatophagoides farinae, Dermatophagoides pteronyssinus, cow's milk, egg white, egg yolk, soy, nut, peanut, wheat, and tuna), with positive and negative controls, histamine and serum physiologic, respectively. Reactions were considered to be positive if an induration >3 mm was observed, compared to the negative control. Total IgE levels were measured with a nephelometric method. Eosinophil counts were determined with Coulter counter leukocyte measurements. Serum ANXA2 levels were measured using ELISA kit (Wuhan USCN Business Company, SEB944HU, Hubei, China), according to the manufacturer's instructions

Data were analyzed using SPSS version 22 software (IBM, USA). The Shapiro-Wilk test was carried out to determine the normality of data distribution and revealed abnormal data distributions for ANXA2 levels, total IgE, and eosinophil counts (P<0.05). Hence, median values and minimum-maximum ranges were determined and all groups were compared using the Kruskal-Wallis test. We also used a post-hoc Bonferroni modified Mann-Whitney U test for binary comparison. Correlation analysis was performed with Spearman's correlation test. Results with p-values <0.05 were considered statistically significant.

This study was approved by the ethic comity of Batman District State Hospital in Batman, Turkey. Written informed consent was acquired from the parents of all participating children.

Results

Of 50 patients with AD, 25 (50 %) were SPT-Pos (aged 3-24 months with median age 8 months, 13 males, and 12 females), 25 were SPT-Neg (aged 3-24 months with median age 11 months, 11 males, and 14 fe¬males). Twenty-seven children were enrolled as a control group (aged 3-34 months with median age of 12 months, 14 males, and 13 females). The demographic characteristics of children participating in our study are shown in Table 1.

Serum median ANXA2 levels were significantly lower in the disease group than in the control group [0.53 (range 0.14 - 4.38) pg/mL vs 0.94 (range 0.40 - 8.74) pg/mL, respectively; p = 0.01]. Levels of ANXA2 were found to be lower in SPT-Pos patients than in SPT-Neg patients [0.45 (range 0.14 - 2.98) pg/mL vs 0.76 (range 0.31 - 4.38) pg/mL, respectively; p = 0.01]. In addition, ANAXA2 levels were found to be lower in SPT-Pos patients than in controls [0.45 (range 0.14 - 2.98) pg/mL vs 0.94 (range 0.40 - 8.74) pg/mL, respectively; p = 0.01]. Lastly, we compared ANXA2 levels between SPT-Neg patients and controls. No significant differences were found between two groups [0.76 (range 0.31 - 4.38) pg/mL vs 0.94 (range 0.40 - 8.74) pg/mL, respectively; p = 0.36] (Table 1 and Figure 1).

When median IgE levels were compared, there was a statistically significant increase in disease group (SPT-Pos patients + SPT-Neg patients) compared to control group [226.5 (range 50 – 1284) IU/mL vs 140 (range 3 – 450) IU/mL, respectively; p=0,002]. Median IgE levels were significantly higher in the SPT-Pos group compared to the control group. [210 (range 76 -1284) IU/mL vs 140 (range 3 - 450) IU/mL, respectively; p= 0.014]. Also, there was a statistically significant increase in the SPT-Neg group compared to the control group for median IgE levels [230 (range 50 - 710) IU/mL vs 140 (range 3 - 450) IU/mL, respectively; p = 0.006]. However, no difference was found between SPT-Pos patients and SPT-Neg patients in terms of IgE levels [210 (range 76 - 1284) IU/mL vs 230 (range 50 - 710) IU/mL, respectively; p = 0.985].

Median eosinophil counts were significantly higher in the disease group than in the controls [370 (range 50 -1640) /mL vs 180 (range 4 – 740) /mL, respectively; p = 0.001]. As expected, eosinophil count in the SPT-Pos group was higher than in the SPT-Neg group [420 (range 120 – 1640) vs 260 (range 50 – 710 /mL) /mL, respectively; p = 0.015]. Also, eosinophil counts were remarkably higher in the SPT-Pos group than in the control group [420 (range 120 – 1640) /mL vs 180 (range 4 – 740) /mL, respectively; p <0.001]. Conversely, no difference was found in eosinophil counts between the SPT-Neg group and the controls [260 (range 50 – 710) /mL vs 180 (range 4 – 740 /mL), respectively; p = 0.068].

In the disease group (SPT-Pos group + SPT-Neg group), no correlation was found between eosinophil counts and IgE levels

(r=0,15, p=0,283), between IgE levels and ANXA2 levels (r= -0,070, p=0,630), and between eosinophil counts and AXA2 levels (r= -0,076, p=0,601).

Also, in SPT-Pos group and SPT-Neg group, no correlation was found among the parameters, between eosinophil counts and IgE levels (r=0,203, p=0,330 and r=0,168p=0,422), between IgE levels and ANXA2 levels (r= -0,047, p=0,825 and r= -0,157 p=0,454), and between eosinophil counts and ANXA2 levels (r= -0,045, p=0,830 and r=0,112 p=0,596), respectively.

Table 1. Patient characteristics, median values of age, IgE and eosinophil count, and ANXA2

Characteristics	SPT-Pos group n=25	SPT-Neg group n=25	Control group n=27
Gender			
Female	12	14	13
Male	13	11	14
Median age (range) months	8 (3 - 28)	11 (3 - 24)	12 (3 - 24)
Median total IgE (range), IU/mL	210 (76 - 1284)	230 (50 - 710)	140 (3 - 450)
Median eosinophil (range), n/mL	420 (120 - 1640)	260 (50 - 710)	180 (4 - 710)
Median ANXA2 (range), pg/mL	0,45 (0.14 - 2,98)	0.76 (0.31 - 4,38)	0.94 (0.40 - 8,74)

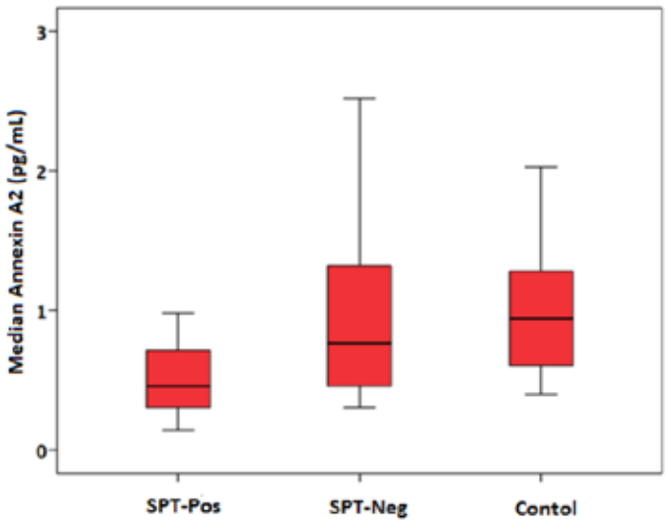

Figure 1. Decreased Annexin A2 (ANXA2) in children with atopic dermatitis

Discussion

Atopic dermatitis, allergic rhinitis, and asthma are common allergic conditions with high morbidity. To our knowledge, no one has investigated a possible role of serum ANXA2 in the pathogenesis of AD in children. In our study, serum ANXA2 levels were significantly lower in AD patients than in controls, especially in the SPT-Pos group. Chronic inflammation is one of the most important characteristics of patients with AD. Like other family members of ANXs, ANXA2 is a pleiotropic protein and is involved in diverse cellular processes, such as cell motility, endocytosis, fibrinolysis, ion channel formation, and as well as cell anti-inflammatory effects [5,9]. In murine models, it was shown that the depletion of ANXA2 down-regulated the production of IL-10 which can cause pro-inflammatory responses. Namely, ANXA2 is required for the optimal production of IL-10 which is an important anti-inflammatory cytokine [9]. Also, ANXA2 reduces plasmin-stimulated IL-6 production and bacteria-induced pulmonary inflammation [9,10]. In a publication, an in vivo murine model was used to examine the potential role of ANXA2 in allergic airway inflammation. In this study, it was shown that ANXA2 gene deletion reduces allergen-induced airway inflammation [10]. In this study, serum ANXA2 levels were lower in AD patients than in controls, especially in the SPT-Pos group which suggests a potential role of ANXA2 in the pathogenesis of AD. To our knowledge, one publication has been reported the ANXA2 role in patients with AD in the medical literature. In that publication, in contrast to our findings, ANXA2 levels were higher in patients with AD than in the controls in the AD lesions [11]. These different findings indicate that future studies are needed on this interesting molecule (ANXA2) in allergic diseases.

In conclusion, the lower levels of ANXA2 may reflect poor responses to chronic inflammatory processes in patients with AD. As known, there is no curative therapy for chronic allergic inflammation in patients with AD. For this purpose, new therapeutic approaches are needed to treat allergic diseases. The ANXA2-associated pathways may be considered in the development of novel therapeutic approaches for the treatment of patients with AD.

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All authors contributed to designing the study, collecting and analyzing the data, writing, and revising the manuscript.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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